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Comparison of culture, polymerase chain reaction (PCR), TaqMan *Salmonella*, and Transia Card *Salmonella* assays for detection of *Salmonella* spp. in naturally-contaminated ground chicken, ground turkey, and ground beef[☆]

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Abstract

Four types of assays were evaluated for the detection of *Salmonella* spp. in retail ground chicken (86 packages), ground turkey (104 packages), and ground beef (54 packages). Two 25 g samples from each package were separately subjected to pre-enrichment in buffered peptone water for 20 h at 37 °C followed by enrichment in Rappaport Vassiliadis (RV) broth for 20 h at 42 °C. The RV enrichments were plated onto Rambach agar, Rainbow Agar *Salmonella*, and XLT4 agar, and were also tested by a PCR assay targeting the *Salmonella invA* gene, as well as by the TaqMan[®] *Salmonella* PCR assay. Additionally, the RV enrichments were tested using the Transia Card *Salmonella*[®] immunoassay. Results showed that 16.8, 24.0, 28.8, and 26.4% of turkey samples were positive for *Salmonella* spp. by culture, PCR, TaqMan PCR, and Transia Card *Salmonella* assays, respectively. Eighteen, 28.5, 35.5, and 34.9% of chicken samples were positive by culture, PCR, TaqMan PCR, and Transia Card *Salmonella* assays, respectively, and 6.5, 6.5, 6.5, and 18.5% of ground beef samples were positive by the four assays, respectively. Analysis of the data using the kappa statistic showed that there was substantial to excellent agreement between the PCR and TaqMan PCR assays and between the PCR and culture assays (kappa coefficients ranging from 0.67 to 0.87), while there was poor to fair agreement between the results of the Transia Card *Salmonella* assay and the other methods (kappa coefficients ranging from 0.28 to 0.32). Overall, results showed that the PCR-based assays were more sensitive than the culture method, and the culture and PCR-based assays were more specific than the immunoassay for detection of *Salmonella* in ground chicken, turkey, and beef due to the occurrence of false positive results using the immunoassay.
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1. Introduction

Nontyphoidal *Salmonella* spp. cause an estimated 1.34 million cases of food-borne illness and 553 deaths per year in the US [1]. *Salmonella* spp. are found in the gastrointestinal tracts of a wide range of animals, thus contact with animals and foods of animal origin are frequent causes of salmonellosis [2,3]. Furthermore, seafood and produce are also documented vehicles of transmission of *Salmonella* to humans [3–6]. Due to the relatively high prevalence of *Salmonella* spp. in meat, poultry, and other foods [7], as well as the high incidence of disease caused by these organisms, rapid, sensitive, and reliable methods for detection of *Salmonella* in foods are needed to reduce the occurrence of salmonellosis. To address the problem of *Salmonella* contamination of raw meat and poultry products, the USDA Food Safety and Inspection Service (FSIS) issued the 'Pathogen Reduction; Hazard Analysis and Critical Control Point (PR/HACCP) Systems; Final Rule' on July 25, 1996 setting *Salmonella* performance standards that the food industry must meet [8]. Testing results of 735, 3,192, and 50,515 randomly-collected ground chicken, ground turkey, and ground beef samples,

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respectively, collected by the USDA FSIS from January 26, 1998 to December 31, 2000 from large, small, and very small establishments showed decreased *Salmonella* prevalences compared to prevalences as determined from the nationwide baseline studies conducted before the PR/HACCP was implemented [9].

It is essential that methods for detection of *Salmonella* and other pathogens in foods have the ability to detect low levels of pathogens that are healthy, as well as those that are stressed/injured due to conditions in the food and/or during food processing. Conventional culture-based methods that rely on enrichment of the food sample and plating onto selective agar media may not be as sensitive as immunologic- or genetic-based 'rapid methods' [10]. Immunologic methods rely on binding of an antigen on the bacterium to a specific monoclonal or polyclonal antibody, and several immunoassay-based systems and devices are commercially available for detection of a variety of food-borne pathogens including *Salmonella* [11,12]. The DNA of the pathogen is the target for nucleic acid-based systems such as probe hybridization or the polymerase chain reaction (PCR). Methods based on the PCR such as the fluorogenic $5'$ nuclease assay offer the advantages of high specificity and sensitivity, and a number of PCR-based kits are commercially available for testing of food or other types of samples for the presence of *Salmonella* or other pathogens [13–15]. The objective of this study was to compare a traditional culture method, PCR-based methods, and a commercially-available immunochromatographic assay for detection of

Salmonella spp. in retail ground chicken, ground turkey, and ground beef.

2. Materials and methods

2.1. Enrichment of ground chicken, turkey, and beef samples

Packages of ground chicken ($n = 86$), ground turkey ($n = 104$), and ground beef ($n = 54$) were purchased from local grocery stores from July 1999 to October 2001. Samples were processed on the day of purchase or after 1 day of storage at 4 °C. The procedure used for enrichment and detection of *Salmonella* in the ground meat products is shown in Fig. 1. Two 25 g samples were removed aseptically from each package of meat and added to 225 ml of sterile buffered peptone water (BPW; GENE-TRAK Systems, Hopkinton, MA) in 500 ml volume flasks and were incubated for 22 h at 37 °C at 150 rpm. The secondary enrichment consisted of adding 500 μl of the BPW enrichment to 9.5 ml of Rappaport Vassiliadis (RV; GENE-TRAK Systems) broth, and the tubes were incubated for 22 h at 42 °C at 150 rpm.

2.2. Detection and isolation of Salmonella spp. on selective media

The enrichments were diluted in sterile 0.1% peptone (Difco, Detroit, MI) and plated using a Spiral Plater (Model
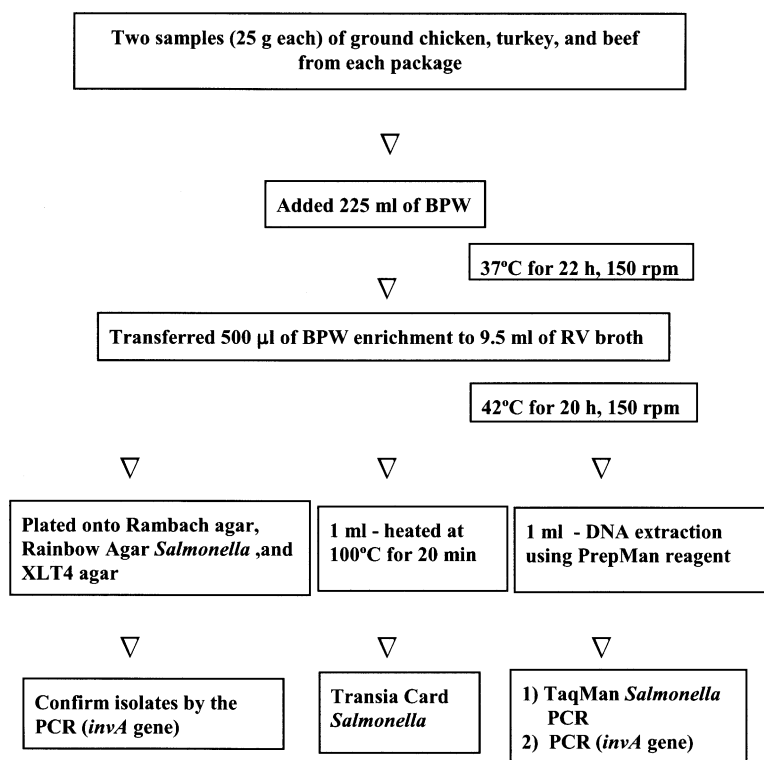

Fig. 1. Procedure used for detection of *Salmonella* in ground chicken, ground turkey, and ground beef.

D, Spiral Biotech, Bethesda, MD) onto Rambach® agar (EM Science, Gibbstown, NJ), Rainbow® Agar *Salmonella* (Biolog, Hayward, CA), and XLT4 agar (XLT4 Agar Base containing the XLT4 agar supplement, 7-ethyl-2-methyl-4-undecanol hydrogen sulfate, sodium salt) (Difco). The plates were incubated at 37 °C for 20 h. Black colonies on Rainbow Agar *Salmonella* and XLT4 agar, and red colonies on Rambach agar were picked and mixed in 50 μl of sterile water. The bacteria were lysed at 99 °C for 10 min in a GeneAmp PCR System 9600 thermal cycler (PE Applied Biosystems, Foster City, CA), then confirmed as *Salmonella* by the PCR by amplifying a portion of the *invA* gene as described below. Selected isolates were sent to the *Salmonella* Reference Center, University of Pennsylvania, New Bolton Center, Kennett Square, PA for serotyping.

2.3. DNA extraction and detection of Salmonella by the PCR and TaqMan® Salmonella assays

One milliliter of the RV enrichment was centrifuged at $16,000 \times g$ for 2 min, and DNA extraction was performed on the cell pellet using the PrepMan reagent (PE Applied Biosystems) following the manufacturer's instructions. The PCR mixture for amplification of the *Salmonella invA* gene consisted of 1 μM of primers INVAF and INVAR, 5′CGGTGGTTTTAAGCGTACTCTT-3′ and 5′-CGAA-TATGCTCCACAAGGTTA-3′, respectively, [16] 20 mM Tris–HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl$_2$, 0.2 mM (each) of the 4 deoxynucleotide triphosphates, and 1.25 U *Taq* DNA polymerase (PCR Reagent System; Invitrogen, Carlsbad, CA). Forty-five microliters of the PCR mixture were added to 5 μl of template DNA, and the PCR was performed on a GeneAmp thermal cycler. The thermal cycling protocol consisted of 94 °C for 2 min, followed by 35 cycles of 94 °C for 20 s, 57 °C for 1 min, and 72 °C for 1 min, with a final extension of 72 °C for 10 min. The PCR yielded a 796 bp product, visualized following electrophoresis on 1.5% agarose gels that were stained with ethidium bromide. Additionally, 5 μl of template DNA were used for the PCR employing the TaqMan *Salmonella* PCR Amplification/Detection Kit (PE Applied Bioystems) following the procedure recommended by the manufacturer.

2.4. Detection of Salmonella by the Transia Card Salmonella® assay

One milliliter of the RV enrichment was heated at 100 °C for 20 min, and 4 drops were placed into the sample well of the Transia Card *Salmonella* test device (GENE-TRAK Systems, Hopkinton, MA). After incubation at room temperature for 10 min, the results were recorded as positive if two reddish-purple lines appeared, one in the test window and one in the control window, and as negative if a line appeared only in the control window.

2.5. Statistical analyses

The results from the four assays were analyzed to test the agreement among methods. The four methods were treated as 'raters', and the simple kappa statistic was calculated to test how well the methods agreed in classifying the samples as positive or negative [17]. In general, values of kappa larger than 0.75 are indicative of excellent agreement beyond chance, whereas values of kappa between 0.40 and 0.75 are indicative of fair to good agreement beyond chance.

3. Results and discussion

The enrichment protocol used for detection of *Salmonella* in retail ground chicken, turkey, and beef samples consisted of a non-selective enrichment in BPW to allow recovery of injured/stressed *Salmonella* [18], followed by a secondary selective enrichment in RV broth. Studies have shown that overall recovery of *Salmonella* from foods and animal feed using RV medium for enrichment was comparable to or higher than recovery using tetrathionate or selenite cysteine broths [19–21]. Interestingly, in one study, RV medium prepared from individual ingredients yielded higher numbers of *Salmonella*-positive samples compared to commercially-prepared RV medium [19]. Furthermore, enrichment in RV at 42 °C was superior to 37 °C when fecal or food samples were pre-enriched in BPW or other media [19,21]. The increased recovery of *Salmonella* in RV at 42 °C compared to 37 °C is due to an increased inhibition of background microflora at the higher temperature [19–21].

Dilutions of the RV enrichments were plated onto Rainbow Agar *Salmonella*, XLT4 agar, and Rambach agar. Rambach agar is based on the metabolism of propylene glycol by *Salmonella*, and typical colonies appear bright red [22]. On Rainbow Agar *Salmonella* and XLT4 agar, *Salmonella* appear as black colonies due to the production of H$_2$S. The XLT4 medium was the most suitable for detection/isolation of *Salmonella* by the culture method compared to Rainbow Agar *Salmonella* or Rambach agar. After incubation for 20 h at 37 °C, the colonies on XLT4 were smaller than those on Rambach agar or Rainbow Agar *Salmonella*, thus there was less crowding from background colonies, rendering isolation of typical black colonies easier. The PCR results showed that only *Salmonella* colonies were black on XLT4 agar, whereas isolation of *Salmonella* from Rainbow Agar *Salmonella* was more problematic, since bacteria other than *Salmonella* also formed black colonies. In addition, non-*Salmonella* bacteria also formed dark pink to red colonies on Rambach agar. Thus XLT4 agar was sufficiently selective, and made it possible to easily differentiate *Salmonella* from other bacteria. This is in agreement with results of a study by Miller et al. [23] showing that XLT4 inhibited *Proteus*, *Pseudomonas*, *Providencia*, and many other non-salmonellae, in addition to providing good
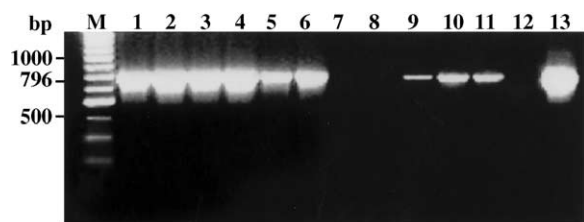
Fig. 2. Analysis of *invA* PCR products (796 bp) from enrichments of 6 different ground chicken samples and 6 different ground turkey samples by agarose gel electrophoresis. Lanes 1 and 2, 7 and 8, and 11 and 12, PCR products from DNA from ground chicken enrichments; lanes 3–6 and lanes 9 and 10, PCR products from DNA from ground turkey enrichments. Lanes 7, 8, and 12 show a negative result for the *invA* PCR product. Lane 13, positive control, DNA from *Salmonella* Typhimurium LT2; lane M, 100 bp ladder molecular weight standards (Invitrogen, Carlsbad, CA).

differentiation between *Salmonella* spp. and *Citrobacter*. In all cases, *Salmonella* was isolated from XLT4 agar, except in one case (ground turkey sample) in which *Salmonella* was isolated from both Rambach agar and Rainbow Agar *Salmonella*, but not from XLT4 agar.

Since two 25 g portions were tested from each package, and on several occasions, only one of the samples was positive (discussed below), each 25 g sample was analyzed as a separate sample. Thus, there were 488 total samples tested. Results showed that 16.8, 24.0, 28.8, and 26.4% of the turkey samples were positive for *Salmonella* spp. by culture, PCR, TaqMan *Salmonella* PCR, and Transia Card *Salmonella* assays, respectively. Eighteen, 28.5, 35.5, and 34.9% of the chicken samples were positive by culture, PCR, TaqMan *Salmonella* PCR, and Transia Card *Salmonella* assays, respectively, and 6.5, 6.5, 6.5, and 18.5% of the ground beef samples were positive by the four assays, respectively. For 17 out of 84 of the ground chicken packages, only one of the two samples produced a positive result by one or more of the assays used, while the other sample from the package produced negative results with all four assays. For six of the 17 packages, only the Transia Card *Salmonella* assay gave a positive result. For 12 out of 104 of the ground turkey packages, only one of the two samples produced a positive result by one or more of the assays tested, while the other sample from the package produced negative results for all four assays. For one of the 12 packages, only the Transia Card *Salmonella* assay produced a positive result. And finally, in two out of 54 ground beef packages, a positive result was obtained for one sample by one or more of the assays tested, while the other sample from the package produced negative results for all four assays. Both of these samples were positive for *Salmonella* by the Transia Card *Salmonella* assay. These results indicate that *Salmonella* are not homogeneously distributed within ground beef and ground poultry, and that more than one sample or a sample larger than 25 g may need to be tested to obtain a positive result.

In agreement with the results in the current study, a number of reports have demonstrated that the PCR is as

sensitive or more sensitive than the traditional culture methods and allows for more rapid detection of pathogens from food and fecal samples [13,14,24–26]. Furthermore, an advantage of the TaqMan-based PCR assay or real-time PCR systems is the absence of post-PCR sample handling, preventing potential contamination due to PCR product carryover, and resulting in more rapid analysis and higher throughput capability. The PCR results for 12 of the samples of ground chicken or turkey are shown in Fig. 2. Samples in lanes 11 (sample No. 119; positive for the *invA* gene product) and 12 (sample No. l20; negative) were from enrichments from two ground chicken samples removed from the same package. Sample No. 119 produced positive results for the PCR (*invA*), TaqMan PCR, and the Transia card assays, but was negative by culture. Sample No.120 was negative by all four assays.

In Table 1, the frequency or the number of times that a particular combination of assay results was obtained is shown. For example, for combination number 1, there were 290 samples in which all types of assays evaluated produced a negative result (0, 0, 0, 0). For combination number 11 (1, 1, 1, 1), there were 43 samples in which the four assays were simultaneously positive, and for combination number 10, there were 26 samples in which only the cultural method, PCR, and TaqMan PCR gave positive results, while results for the Transia Card *Salmonella* assay were negative (1, 1, 1, 0). By summarizing the data in this manner, it is readily apparent that differences in sensitivity and specificity among different types of detection methods exist.

In Tables 2–4, the kappa values for each two by two table pairing of the four methods for the results for the chicken, turkey, and beef samples, respectively, are

Table 1
Summary of results for assays for detection of *Salmonella* spp. in ground chicken, ground turkey, and ground beef

Observed combination	Cultural method	PCR	TaqMan *Salmonella*	Transia Card *Salmonella*	Frequency
1	0[a]	0	0	0	290
2	0	0	0	1[b]	72
3	0	0	1	0	17
4	0	0	1	1	2
5	0	1	0	0	1
6	0	1	1	0	19
7	0	1	1	1	14
8	1	0	1	0	1
9	1	0	1	1	2
10	1	1	1	0	26
11	1	1	1	1	43
Total number of samples					488

The two samples tested from each package were recorded as separate samples, thus results are of 488 samples rather than of the 244 packages of meat.
[a] 0 = Negative result.
[b] 1 = Positive result.

Table 2
Kappa statistic values showing agreement between the cultural method, the PCR (*invA* gene), TaqMan *Salmonella*, and Transia Card *Salmonella* assays for ground chicken

	Cultural method	PCR	TaqMan *Salmonella*	Transia Card *Salmonella*
Cultural method	–	0.6824*	0.6103*	0.2643*
PCR	0.6824*	–	0.8460*	0.1563*
TaqMan *Salmonella*	0.6103*	0.8460*	–	0.1256
Transia Card *Salmonella*	0.2643*	0.1563*	0.1256	–

* Indicates that kappa is significantly ($p < 0.05$) nonzero.

shown. Overall, analysis of the results for three types of foods using the simple kappa statistic showed excellent agreement between the PCR and TaqMan *Salmonella* PCR assays, and good to excellent agreement between the PCR and the cultural methods. The agreement between the TaqMan *Salmonella* PCR and the cultural method was good but not as strong. The agreement between the PCR and TaqMan *Salmonella* PCR was excellent. There was poor agreement between the Transia Card *Salmonella* assay and the other three assays, indicating that the Transia Card *Salmonella* assay produced false positive or negative results in many cases. The data do not show differences in performance of the four methods for detection of *Salmonella* based on the type of meat.

A report describing serotyping results of *Salmonella* isolates recovered from cattle, swine, and poultry carcasses and from raw ground product samples collected from different establishments by the USDA FSIS from June 1997 to August 1998 prior to implementation of the PR/HAACP Systems final rule, showed that *Salmonella* Heidelberg, Kentucky, Hadar, and Typhimurium were the most common

serotypes isolated from chicken carcasses, and Heidelberg, Kentucky, Schwarzengrund, and Infantis were the most common isolated from raw ground chicken, with Heidelberg being the most common from both carcasses and raw product [27]. *Salmonella* Hadar, Heidelberg, Agona, and Senftenberg were the most common serotypes isolated from turkey carcasses, and Hadar, Agona, Muenster, and Senftenberg were the most common isolated from raw ground turkey, with Hadar being the most common isolated from carcasses and raw product. *Salmonella* Montevideo was the most common serotype isolated from cattle carcasses and also the most common isolated from raw ground beef. Bailey et al. [28] found that *Salmonella* Senftenberg was a predominant serotype isolated from chicken hatchery samples and from the processing plant. They concluded that hatchery disinfection should be an important component of intervention programs for broiler production operations. The serotypes of selected isolates recovered in the current study from XLT4 agar plates are shown in Table 5. *Salmonella* Heidelberg, Typhimurium, and Kentucky were serotypes isolated from chicken in

Table 3
Kappa statistic values showing agreement between the cultural method, the PCR (*invA* gene), TaqMan *Salmonella*, and Transia Card *Salmonella* assays for ground turkey

	Cultural method	PCR	TaqMan *Salmonella*	Transia Card *Salmonella*
Cultural method	–	0.7333*	0.6496*	0.3160*
PCR	0.7333*	–	0.8516*	0.4343*
TaqMan *Salmonella*	0.6496*	0.8516*	–	0.3700*
Transia Card *Salmonella*	0.3160*	0.4343*	0.3700*	–

* Indicates that kappa is significantly ($p < 0.05$) nonzero.

Table 4
Kappa statistic values showing agreement between the cultural method, the PCR (*invA* gene), TaqMan *Salmonella*, and Transia Card *Salmonella* assays for ground beef

	Cultural method	PCR	TaqMan *Salmonella*	Transia Card *Salmonella*
Cultural method	–	1.0000*	1.0000*	0.3042*
PCR	1.0000*	–	1.0000*	0.3042*
TaqMan *Salmonella*	1.0000*	1.0000*	–	0.3042*
Transia Card *Salmonella*	0.3042*	0.3042*	0.3042*	–

* Indicates that kappa is significantly ($p < 0.05$) nonzero.

Table 5
Serotypes of *Salmonella* strains isolated from ground chicken, ground turkey, and ground beef

Sample type	Group	Serotype
Ground chicken	B	*Salmonella* Heidelberg
	E2	*Salmonella* Newington
	B	*Salmonella* Typhimurium
	C3	*Salmonella* Kentucky
	B	*Salmonella* Typhimurium
	B	*Salmonella* Heidelberg
Ground turkey	B	*Salmonella* Brandenburg
	B	*Salmonella* Schwarzengrund
	B	*Salmonella* Schwarzengrund
	B	*Salmonella* Heidelberg
	B	*Salmonella* Heidelberg
	B	*Salmonella* Saint paul
	B	*Salmonella* Saint paul
	B	*Salmonella* Reading
Ground beef	K	*Salmonella* Cerro
	B	*Salmonella* Typhimurium
	E1	*Salmonella* Anatum
	C1	*Salmonella* Infantis

Only a portion of the *Salmonella* isolates recovered from the ground chicken, turkey, and beef samples were serotyped.

agreement with Schlosser et al. [27], and *Salmonella* Schwarzengrund and Heidelberg were isolated from turkey. None of the isolates was *Salmonella* Senftenberg. Only a few isolates from the ground products were serotyped; therefore a larger study is warranted to determine *Salmonella* serotypes associated with different types of meat.

In conclusion, several different serotypes of *Salmonella* were isolated from retail ground chicken, turkey, and beef samples. Overall, results showed that the PCR-based assays were more sensitive than the culture method, and the culture and PCR-based assays were more specific than the immunoassay for detection of *Salmonella* in these foods. The poor specificity of the immunoassay is likely due to poor performance of the antibody used in the device. The percentage of positive samples obtained by the TaqMan *Salmonella* PCR assay was 35.5, 28.8, and 6.5 for the ground chicken, ground turkey, and ground beef, respectively. The overall prevalence of *Salmonella*-positive ground chicken, turkey, and beef samples collected from establishments of all sizes from January 26, 1998 to December 31, 2000 was 14.4, 29.7, and 3.7%, respectively, [9] which are somewhat lower than results obtained in the current study for ground chicken and ground beef using the TaqMan *Salmonella* assay. Rose et al. [9] tested one 25 g sample collected prior to packaging, and the enrichment was screened using a commercially-available immunoassay then confirmed by culture. In the current study, in a number of cases, only one of the two samples from each package was positive for *Salmonella* by any of the assays employed. Thus to increase the probability of finding a positive sample

it is important to test a portion larger than 25 g and/or to take portions from multiple sites in the food. Use of PCR-based systems may also enhance the ability to detect *Salmonella* in foods.

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